

# Trehalose Toxicity in *Cuscuta reflexa*<sup>1</sup>

## CORRELATION WITH LOW TREHALASE ACTIVITY

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### ABSTRACT

A toxic effect of  $\alpha,\alpha$ -trehalose in an angiospermic plant, *Cuscuta reflexa* (dodder), is described. This disaccharide and its analogs, 2-aminotrehalose and 4-aminotrehalose, induced a rapid blackening of the terminal region of the vine which is involved in elongation growth. From the results of *in vitro* growth of several angiospermic plants and determination of trehalase activity in them, it is concluded that the toxic effect of trehalose in *Cuscuta* is because of the very low trehalase activity in the vine. As a result, trehalose accumulates in the vine and interferes with some process closely associated with growth. The growth potential of *Lemna* (a duckweed) in a medium containing trehalose as the carbon source was irreversibly lost upon addition of trehalosamine, an inhibitor of trehalase activity. It is concluded that, if allowed to accumulate within the tissue, trehalose may be potentially toxic or inhibitory to higher plants in general. The presence of trehalase activity in plants, where its substrate has not been found to occur, is envisaged to relieve the plant from the toxic effects of trehalose which it may encounter in soil or during association with fungi or insects.

$\alpha,\alpha$ -Trehalose ( $\alpha$ -D-glucopyranosyl- $\alpha$ -D-glucopyranoside) is widely distributed in actinomycetes, fungi, insects, and other invertebrates (4). In photosynthetic plants, this sugar has been reported to occur in algae (19) and pteridophytes (14, 26). Reports on the occurrence of trehalose in higher plants (15, 23) has been repudiated by Gussin (10) as due to faulty techniques and microbial contamination and he has also concluded that trehalose does not occur in the angiosperms. However, trehalase (EC 3.2.1.28) activity has been found in all higher plants examined (1, 6, 8, 11, 12). A variety of plant tissues are able to utilize trehalose for growth *in vitro* (5, 12, 20, 22, 27), though the growth response is poor in some cases (30).

This paper reports the toxic effect of trehalose on certain higher plants, an observation hitherto not reported. This observation was made when various carbohydrates were tested as energy sources for the *in vitro* growth of the excised shoot tip of dodder, *Cuscuta*, as an indeterminate meristem (18). The *in vitro* culture technique was therefore used to study the responses of several higher plant tissues to trehalose to determine the extent to which this disaccharide is toxic to angiospermous plants. The results suggest that trehalose may be toxic to those plant tissues where trehalase activity is low. The implications of these findings are discussed.

### MATERIALS AND METHODS

**In Vitro Culture of Plants.** Dodder shoot tips were cultured as described previously (18). This species of dodder, erroneously

described as *Cuscuta chinensis* Lamk. (18), has now been identified as *Cuscuta reflexa* Roxb. The culture medium was the same as used earlier (18), except that the medium lacking a sugar is designated as BM.<sup>3</sup>

Callus cultures were generated from *Datura innoxia* Linn. (stem), *Nicotiana tabacum* Linn. var. Wisconsin 38 (stem), *Daucus carota* Lamk. (root) and *Glycine max* Linn. (hypocotyl). These calli were initiated and maintained on Murashige and Skoog's medium (21) supplemented with 3% sucrose,  $10^{-5}$  M  $\alpha$ -naphthaleneacetic acid and  $10^{-5}$  M BA and solidified with 0.8% (w/v) Difco agar. The cultures were grown in tubes (15 × 2.5 cm) containing 10 ml medium and were subcultured at intervals of 20 days.

Shoot explants were obtained from 7- to 14-day-old seedlings of *Zea mays* Linn., *Raphanus sativus* Linn., *Quamoclit phoenicea* Choisy, and *Phaseolus radiatus* Linn. The seedlings were grown aseptically in BM solidified with 0.8% (w/v) agar in tubes (20 × 2.5 cm). The roots and cotyledons were removed; the shoots were placed upright in 1 ml liquid BM without or with a sugar (2%, w/v).

*Lemna paucicostata* Heg. strains LP6 and 6746 (obtained from Prof. S. C. Maheshwari, University of Delhi) were cultured in 50 ml Hutner's (2) half strength medium without a carbon source in 100 ml Erlenmeyer flasks. The plants were multiplied by transferring 15 to 20 fronds to new medium at intervals of 30 days.

All cultures were maintained at  $25 \pm 2^\circ\text{C}$  with a 16-h light and 8-h dark regimen. The illumination was from fluorescent lamps (cool-white, 40 w), placed 50 cm from the cultures, which yielded a light intensity of about 150 lux at the level of the cultures.

**Enzyme Assays.** The tissue (1–2 g) was homogenized with 1 g sand and 200 mg insoluble polyvinylpyrrolidone in 5 ml 50 mM sodium acetate buffer (pH 5.5), containing 100 mM sodium ascorbate (17). The crude homogenate was centrifuged at 12,000g for 15 min; the supernatant was dialyzed twice against 1 liter 50 mM sodium acetate buffer (pH 5.5). The dialyzed extract was centrifuged at 12,000g and the clear supernatant used as enzyme.

Trehalase activity in the extract was measured by estimating the glucose produced by the hydrolysis of trehalose by the method of Somogyi (28) or by the glucose oxidase-peroxidase method (7). The reaction mixture contained 0.2 ml of the substrate (20 mM trehalose) and a suitable volume of the extract. The total volume was made up to 2 ml with 50 mM sodium acetate buffer (pH 5.5). The reaction mixture was incubated at  $30^\circ\text{C}$  for 30 min.

Protein in the tissue homogenates was estimated by the method of Potty (24). Specific activity of trehalase is expressed as nmol of glucose formed  $\text{min}^{-1} \text{mg}^{-1}$  protein at  $30^\circ\text{C}$ .

**Paper Chromatography of Tissue-Extracted Sugars.** The tissue chopped into 5-mm pieces was immediately plunged into 15 ml boiling 80% (v/v) ethanol in a 100-ml Erlenmeyer flask, and extracted for 10 to 15 min. The extract was decanted and the tissue was reextracted three times with 15 ml volumes of the solvent.

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<sup>3</sup> Abbreviations: BM, basal medium.

The combined extract was dried under reduced pressure between 40 to 45°C in a rotary film evaporator. The residue, freed of lipids by extracting twice with 15 ml chloroform-methanol (2:1, v/v), was dissolved in 10 ml water and centrifuged. Five ml of the aqueous solution containing the sugars was deionized by successive passage through columns (3.5 × 1.6 cm) of Dowex 50 H<sup>+</sup> form (200–400 mesh) and Dowex 1 HCOO<sup>−</sup> form (200–400 mesh). The columns were washed three times with 5 ml portions of deionized H<sub>2</sub>O. The eluate was evaporated to dryness under reduced pressure between 40 to 45°C. The residue (neutral fraction) was dissolved in water and subjected to descending paper chromatography for 36 to 40 h on Whatman No. 3 paper using 1-butanol:ethanol:water (52:33:15, v/v/v) as the solvent. The sugars were detected by dipping the chromatograms in an alkaline silver nitrate solution (29).

**Estimation of Trehalose.** Trehalose in the neutral extracts was estimated enzymically, using trehalase from a thermophilic fungus, *Thermomyces lanuginosus* Tsiklinski (formerly called *Humicola lanuginosa*), purified by the method of Prasad and Maheshwari (25). This enzyme, purified 138-fold, was free of all other glycosidase activities tested. A mixture of the neutral extract and trehalase (117 units) in 50 mM sodium acetate buffer (pH 5.5) (total volume 1 ml) was incubated for 2 h at 40°C. The reaction was stopped by placing the tubes in a boiling water bath for 5 min. Glucose formed was estimated by the glucose oxidase-peroxidase method (7). Trehalose content was calculated by multiplying the glucose value with 0.95. Total sugar content was estimated by the method of Yemm and Willis (31).

**Chemicals.** Inorganic chemicals, solvents, sucrose, and glucose were of analytical reagent grade. Other biochemicals were bought from Sigma. 2-Aminotrehalose was received as gifts from Prof. Alan D. Elbein, University of Texas Health Science Centre, and Dr. Arcamone, Farmitalia, Italy.  $\alpha,\alpha$ -Allotrehalose,  $\alpha,\alpha$ -galactotrehalose and  $\beta,\beta$ -trehalose were gifts from Dr. C. K. Lee, Tate and Lyle Ltd., England. 4-Aminotrehalose was a gift from Prof. H. Umezawa, Institute of Microbial Chemistry, Tokyo.

## RESULTS

**Effects of Different Sugars on *In Vitro* Growth of *Cuscuta* Shoot Tips.** The shoot tips cultured in BM (no sugar) were green and exhibited some growth (Table I). Mannose and mannitol were poor carbon sources. Fructose, galactose, glucose, lactose, maltose, cellobiose, gentiobiose, isomaltose, raffinose, and melezitose supported good growth and were comparable to sucrose in their effects. In marked contrast,  $\alpha,\alpha$ -trehalose caused blackening of the shoot tips within 3 days. These explants were considered to have been killed.

Several analogs of trehalose were tested for their effect on the shoot tips of *Cuscuta* (Table II).  $\alpha,\alpha$ -Galactotrehalose ( $\alpha$ -D-galac-

topyranosyl- $\alpha$ -D-galactopyranoside) and  $\beta,\beta$ -trehalose ( $\beta$ -D-glucopyranosyl- $\beta$ -D-glucopyranoside) supported growth.  $\alpha,\alpha$ -Allotrehalose ( $\alpha$ -D-allopyranosyl- $\alpha$ -D-allopyranoside) neither supported growth nor was toxic. 2-Aminotrehalose (2-deoxy-2-amino- $\alpha$ -D-glucopyranosyl- $\alpha$ -D-glucopyranoside) and 4-aminotrehalose (4-deoxy-4-amino- $\alpha$ -D-glucopyranosyl- $\alpha$ -D-glucopyranoside) were toxic.

The minimum concentration of  $\alpha,\alpha$ -trehalose required to cause blackening of *Cuscuta* shoot tips was 0.5% (w/v) (Fig. 1). In 2% trehalose, 92% of the explants blackened. This concentration was used in the subsequent experiments. After 3 and 6 days, the blackened explants numbered 50 and 93%, respectively.

**Trehalose Toxicity in Long *Cuscuta* Vines.** Vines of different lengths were fed trehalose for 3 days through their cut ends. These were not surface-sterilized, but their cut ends were rinsed in sterile water and dipped in 0.3 ml sterile medium per tube. Microbial contamination was minimized by daily transfers of the vines into tubes containing fresh medium. In all vines, blackening was confined to the terminal 2.5-cm portion (Fig. 2).

Whether the blackening of the terminal region of the vine was due to the selective accumulation of trehalose in this region was investigated. Vines 10-cm long, marked at 2-cm intervals from the apex, were fed 2% trehalose for 2 days. The different regions of the vine were then analyzed for trehalose. Trehalose was present in all the regions of the vine constituting about 23 to 36% of the

Table II. Effect of Trehalose Analogs on the gibberellic acid A<sub>3</sub>-Promoted Growth of the Shoot Tips of *Cuscuta*

Shoot tip explants were cultured for 7 days in filter-sterilized media (0.3 ml) containing 2% (w/v) sugar and 14.4  $\mu$ M gibberellic acid A<sub>3</sub>. Growth is expressed as difference between the final and initial length (2.5 cm) of the explant. Number of replicates was 3 to 4.

Sugar	Growth cm $\pm$ SE	Explant Color
Control (no sugar)	0.8 $\pm$ 0.1	Green
$\alpha,\alpha$ -Galactotrehalose	2.5 $\pm$ 0.4	Green
$\beta,\beta$ -Trehalose	2.7 $\pm$ 0.1	Green
$\alpha,\alpha$ -Allotrehalose	0.2	Green
$\alpha,\alpha$ -Trehalose	0.2 $\pm$ 0.1	Black
2-Aminotrehalose	0.3 $\pm$ 0.1	Black
4-Aminotrehalose	0.2 $\pm$ 0.1	Black

Table I. Effect of Sugars on Growth of *Cuscuta* Shoot Tips *In Vitro*

All media (1 ml) contained 14.4  $\mu$ M gibberellic acid A<sub>3</sub> and a sugar at 5% (w/v). Growth is expressed as difference between the final and the initial length (2.5 cm) of the explant.

Sugar	Growth Period days	Growth <sup>a</sup> cm $\pm$ SE
Sucrose <sup>b</sup>	17	9.2 $\pm$ 0.5
Mannose	17	2.7 $\pm$ 0.3
Mannitol	17	0.5 $\pm$ 0.04
Trehalose	25	0
No sugar	25	0.4 $\pm$ 0.05

<sup>a</sup> Number of replicates was 19 to 29.

<sup>b</sup> Glucose, fructose, galactose, lactose, maltose, cellobiose, isomaltose, gentiobiose, raffinose, and melezitose supported growth comparable to sucrose.

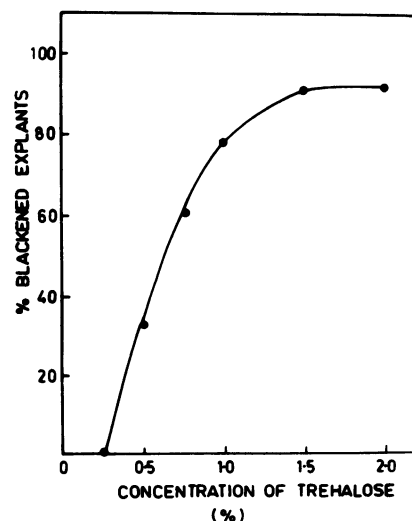


FIG. 1. Dose-response curve of trehalose-induced toxicity in excised shoot tip explants (2.5 cm) of *Cuscuta*. The shoot tip was cultured in 0.3 ml medium. Observations were made after 6 days. Number of replicates per treatment was 18 to 24.

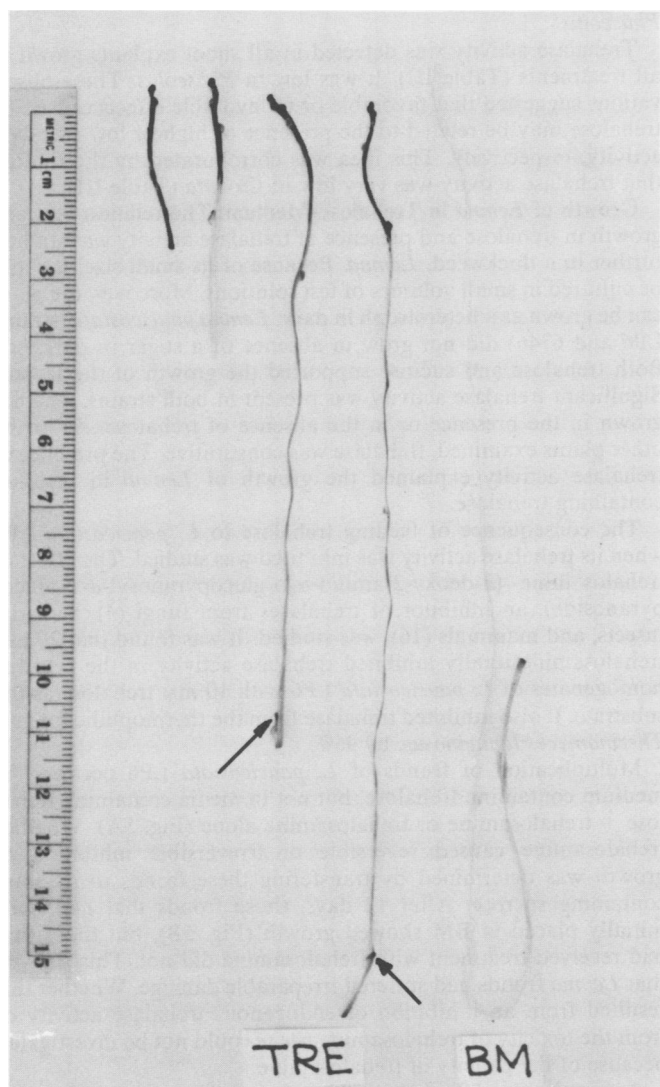


FIG. 2. The effect of trehalose on the excised vines of *Cuscuta*. The vines (2.5, 5, 10, and 15 cm) were placed for 4 days in BM and in BM + 2% trehalose (TRE). Note the blackening of the terminal portions in all the trehalose-fed vines. In the 10- and 15-cm-long vines the basal buds (marked with arrow) had also blackened. Pigments were removed with hot ethanol before the photograph was taken.

total soluble sugars. There was about 50% more trehalose in the 2 to 4 cm region than in the other regions of the vine. The results showed that the localized blackening of the terminal (2.5 cm) region of the vine was not due to a selective accumulation of trehalose at this site.

The site of trehalose toxicity was analyzed with respect to the region of growth in *Cuscuta* vine. Excised vines (15 cm) were marked at intervals of 1 cm from the apex; their cut ends were dipped in (a) water, (b) BM, (c) BM + 2% sucrose, and (d) BM + 2% trehalose. After 4 days, the final length of the vines and the distance between the marks were measured. The growth increment in the vines placed in water, BM or BM + sucrose occurred to almost the same extent, being 80%. The maximum elongation occurred in the terminal 2-cm region of the vine (Fig. 3). In contrast, the growth increment of vines placed in trehalose was only 13%. These vines blackened in the terminal 2.5-cm region and somewhat more. Thus, trehalose feeding resulted in the blackening of that region of the vine which is involved in elongation growth.

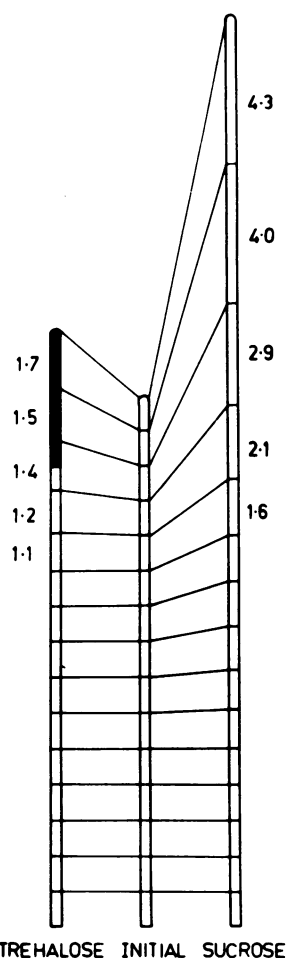


FIG. 3. Diagrammatic relationship between the region of elongation growth and the region of trehalose-induced blackening in *Cuscuta* vine. Initial length of the vine was 15 cm. These vines were marked at 1-cm intervals from the apex. Trehalose or sucrose was fed to the vines at 2% (w/v) for 4 days. Final lengths (cm) of the terminal regions of the vines (initial 5 cm region) are indicated. Values are means of four replicates. Blackened region of the trehalose-fed vine is shaded.

When a 50-cm long vine was fed 2% trehalose, blackening of the apical tissue occurred on the 9th day. Strikingly, a previously dormant lateral bud, which had been released from apical dominance and had started to grow, also blackened. The important point disclosed from this observation was that the actively growing region in *Cuscuta* vine was the site of trehalose toxicity. In an experiment with 15-cm vine, the inhibition of growth was evident as early as 12 h, although the blackening of the vines started only after 36 h (Fig. 4).

A close observation of the trehalose-fed vine revealed that minute droplets appeared in the 0 to 2 cm region prior to blackening. Blackening developed later as a patch at the area of the droplet. Soon after, the entire site turned black. The exudate turned brown and subsequently gelled. The leakage of liquid indicated that the tissue was disintegrated.

Decapitation (removal of 3 mm from the tip) did not prevent the blackening of 2.5-cm shoot tip explant, showing that the toxicity was not dependent upon the presence of the shoot apex. The need for metabolic activity of the tissue for trehalose-mediated toxicity was investigated. The shoot tips were transferred to BM or BM + 2% trehalose and were kept at 4 to 6°C to impose quiescence. Blackening was not observed up to 13 days. Soon after the cultures were transferred to room temperature, trehalose-fed explants blackened, whereas the control explants cultured in BM

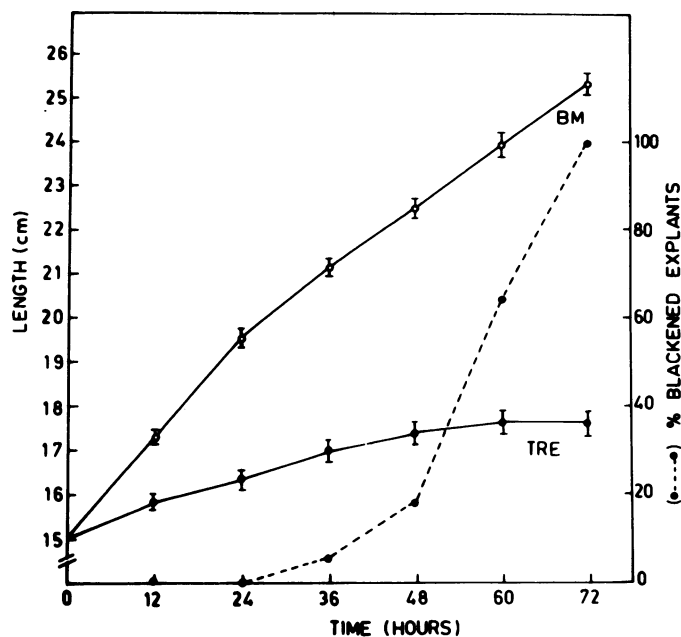


FIG. 4. Growth of 15-cm-long cut vines of *Cuscuta* in BM and in BM + trehalose (TRE). Data represent means of 18 replicates. Bars indicate SE.

remained green. This showed that trehalose-induced blackening in *Cuscuta* was dependent upon metabolic activity.

**In vitro Growth of Plant Tissue Cultures in Media Containing Trehalose.** The observation of the toxic effect of trehalose in *Cuscuta* led to the question of whether trehalose was also toxic to other plant tissues. Therefore, whole plants, organs, and tissue (callus) cultures were tested for their ability to utilize trehalose for growth. The growth of *Nicotiana*, *Datura*, *Daucus*, and *Glycine* tissue cultures in BM (no sugar) was negligible. Growth of all tissues in trehalose was comparable to that in sucrose except for *Glycine* in which it was 30% of that in sucrose. Trehalose-hydrolyzing activity was present in all tissue cultures; the specific activity ranging from 5 to 30. Based on the knowledge from other systems that hydrolysis of trehalose is catalyzed by a highly specific enzyme and that other glycosidases have not been reported to hydrolyze trehalose (6, 12, 16), it is assumed that the trehalose-hydrolyzing activity in plant tissue is due to trehalase (EC 3.2.1.28). Trehalase was synthesized constitutively since it was present in tissue cultures grown in media containing sucrose or trehalose. The ability of trehalose to support growth of these tissue cultures can therefore be related to the presence of trehalase activity in them.

**Growth of Shoot Explants in Trehalose Medium.** Excised shoots of *Zea mays* were transferred into test solutions when the leaves were still within the coleoptiles. A toxic effect of trehalose was not observed. In 6 days, the explants in BM, BM + 2% sucrose, and BM + 2% trehalose developed two to three new leaves and roots. Shoot explants of *Raphanus* and *Quamoclit* had the first pair of small leaves at the beginning of the experiment. In both shoot explants, growth in the absence of sugar (BM) was limited. However, growth of new leaves and roots was observed when fed with sucrose or trehalose. Trehalose, therefore, supported the growth of the shoot explants of *Raphanus* and *Quamoclit* and was not toxic to them.

The primary leaves of *Phaseolus* were folded at the beginning of the experiment. In 3 days, in BM and BM + 2% sucrose, the hypocotyls elongated and the leaves enlarged and unfolded. In BM + 2% trehalose, the elongation growth was reduced and wilting of leaves was observed. Trehalose was thus inhibitory to

#### *Phaseolus*.

Trehalase activity was detected in all shoot explants grown in all treatments (Table III). It was low in *Phaseolus*. These observations suggested that favorable or unfavorable effects caused by trehalose may be related to the presence of high or low trehalase activity, respectively. This idea was corroborated by the finding that trehalase activity was very low in *Cuscuta* (Table III).

**Growth of *Lemna* in Trehalose-Medium.** The relation between growth in trehalose and presence of trehalase activity was studied further in a duckweed, *Lemna*. Because of its small size, it could be cultured in small volumes of test solutions. Moreover, the plant can be grown as a heterotroph in dark. *Lemna paucicostata* (strains LP6 and 6746) did not grow in absence of a sugar in darkness. Both trehalose and sucrose supported the growth of the fronds. Significant trehalase activity was present in both strains, whether grown in the presence or in the absence of trehalose. As in the other plants examined, trehalase was constitutive. The presence of trehalase activity explained the growth of *Lemna* in medium containing trehalose.

The consequence of feeding trehalose to *L. paucicostata* LP6 when its trehalase activity was inhibited was studied. The effect of trehalosamine (2-deoxy-2-amino- $\alpha$ -D-glucopyranosyl- $\alpha$ -D-glucopyranoside), an inhibitor of trehalases from fungi (4), bacteria, insects, and mammals (16), was studied. It was found that 20 mM trehalosamine totally inhibited trehalase activity in the cell-free homogenates of *L. paucicostata* LP6 with 10 mM trehalose as the substrate. It also inhibited trehalase from the thermophilic fungus, *Thermomyces lanuginosus*, by 95%.

Multiplication of fronds of *L. paucicostata* LP6 occurred in medium containing trehalose, but not in media containing trehalose + trehalosamine or trehalosamine alone (Fig. 5A). Whether trehalosamine caused reversible or irreversible inhibition of growth was determined by transferring these fronds to medium containing sucrose. After 12 days, those fronds that had been initially placed in BM showed growth (Fig. 5B), but those that had received treatment with trehalosamine did not. This showed that *Lemna* fronds had suffered irreparable damage. Whether this resulted from an inhibition of endogenous trehalase activity or from the toxicity of trehalosamine *per se* could not be investigated because of the paucity of trehalosamine.

**Accumulation of Trehalose.** The observations suggested that in plants with low trehalase activity, trehalose may accumulate and cause growth inhibition or produce toxic syndrome. To verify this, the presence of trehalose in *Cuscuta*, *Phaseolus*, and *L. paucicostata* LP6 was checked in the neutral fractions of the ethanolic extracts of tissues. Trehalose was not detected in *Cuscuta*, *Phaseolus*, and *L. paucicostata* LP6. However, trehalose was detected in *Cuscuta* and *Phaseolus* after feeding this sugar. It was not detected in *L. paucicostata* LP6 even after trehalose feeding.

Using purified trehalase, it was confirmed that trehalose did not occur naturally in *Cuscuta*, *Phaseolus*, and *L. paucicostata* LP6. When cultured in 2% trehalose, the explants of *Cuscuta* and

Table III. Specific Activity of Trehalase in Shoot Explants Cultured in Media Containing Sucrose or Trehalose

Species	Culture Medium		
	BM	BM + 2% Trehalose	BM + 2% Sucrose
<i>specific activity of trehalase</i> <sup>a</sup>			
<i>Zea mays</i>	5.1	7.9	5.5
<i>Raphanus sativus</i>	10.7	96.3	45.1
<i>Quamoclit phoenicea</i>	23.4	37.5	40.4
<i>Phaseolus radiatus</i>	0.8	1.4	1.1
<i>Cuscuta reflexa</i>	0.3	0.2	0.3

<sup>a</sup> nmol glucose formed min<sup>-1</sup> mg<sup>-1</sup> protein.

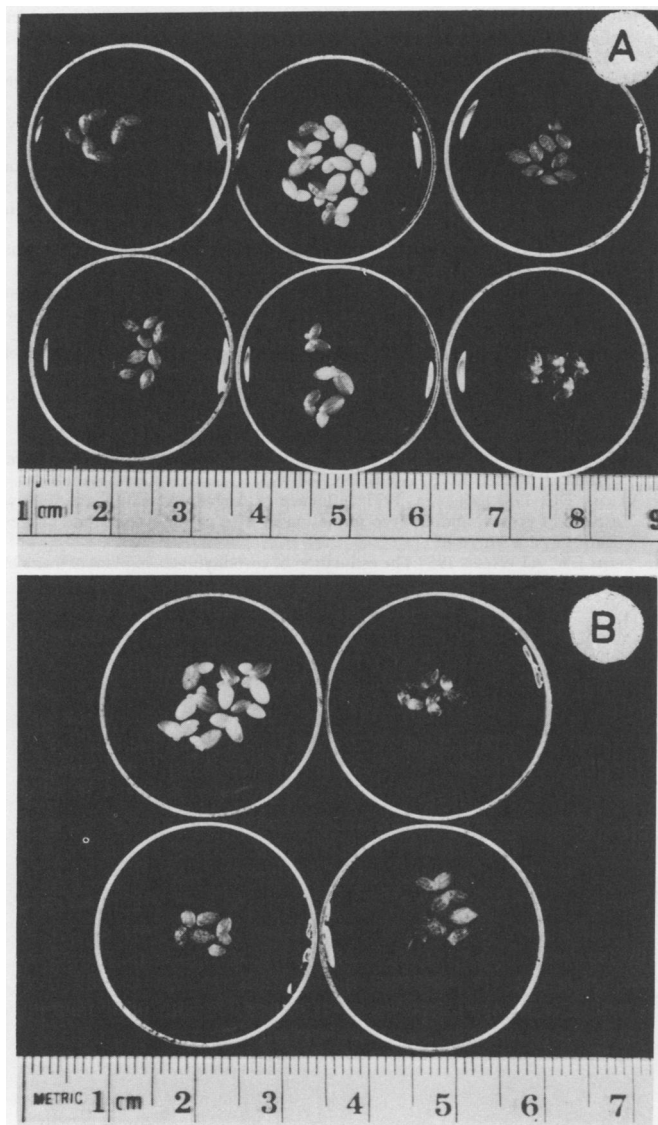


FIG. 5. Culture of *L. paucicostata* LP6 in media containing trehalose and trehalosamine. A, the fronds were cultured for 10 days in dark in the following media: top row, from left—(1) BM, (2) BM + 1% trehalose, and (3) BM + 1% trehalosamine. Bottom row, from left—(1) BM + 1% trehalose + 1% trehalosamine, (2) BM + 1% trehalose + 0.5% trehalosamine, and (3) BM + 1% trehalose + 0.1% trehalosamine. B, after 10 days in media containing trehalose and/or trehalosamine, the fronds were transferred to BM + 1% sucrose and grown in dark for 12 days. The media from which the fronds were transferred are: top row, from left—(1) BM and (2) BM + 2% trehalose + 0.1% trehalosamine. Bottom row, from left—(1) BM + 1% trehalose + 0.5% trehalosamine and (2) BM + 1% trehalosamine.

*Phaseolus* accumulated trehalose to an extent of 29 and 79% of total sugars, respectively. The fronds of *L. paucicostata* LP6, cultured in medium containing trehalose, did not accumulate trehalose. These results showed that those plants which accumulated this sugar exhibited toxic symptoms upon trehalose feeding.

## DISCUSSION

Our observations show the toxic effect of  $\alpha,\alpha$ -trehalose to some higher plants for the first time.  $\alpha,\alpha$ -Trehalose, 2-aminotrehalose, and 4-aminotrehalose were toxic (Table II) indicating the requirement of  $\alpha$ -1,1-linkage of two glucose moieties for causing the

toxicity. The structural specificity required for causing the toxic effect in *Cuscuta* is striking. The trehalose analogs,  $\alpha,\alpha$ -galactotrehalose and  $\beta,\beta$ -trehalose, which supported growth were probably cleaved by nonspecific  $\alpha$ -galactosidase and  $\beta$ -glucosidase, respectively.  $\alpha,\alpha$ -Allotrehalose neither supported growth nor was toxic. This could be due to conformational 'puckering' of allose, causing it to be nonreactive in living tissue.

It is significant that trehalose exerted its toxic effect only in the growing region of the vine (Fig. 2). The observation that the decapitated shoot tip responded to trehalose and turned black indicated that the meristematic cells are not the prime sites of its action. Therefore, trehalose seems to affect the region of cell elongation rather than of cell division in the shoot tip of *Cuscuta*. This situation is similar to the inhibition caused by galactose on cell expansion in tomato roots (13). The specific effect of trehalose on the elongation growth of *Cuscuta* may be potentially useful in clarifying biochemical mechanisms involved in elongation growth in dicotyledonous stems.

The ability of pollen grains of several plants to germinate in medium containing trehalose was correlated with the presence of trehalase activity (12). The present study has established that growth of higher plants in trehalose is related to the presence of trehalase. The plants which showed symptoms of toxicity possessed low trehalase activity (Table III). The observation gives credence to the idea that the toxic effect of trehalose is correlated with a low trehalase activity in plant. The analysis of trehalose content within the tissue after trehalose feeding suggests that, if allowed to accumulate within the plant, trehalose may become potentially toxic or inhibitory to higher plants.

The toxicity of trehalose to higher plants would explain the absence of trehalose in angiosperms. It is possible that the roots of higher plants may encounter trehalose in soil where this sugar may come from the lysis of spores and mycelium of soil fungi. Higher plants could also encounter trehalose during their symbiotic or parasitic association with bacteria, fungi, or insects. In such conditions, trehalase would serve to relieve the plant from the toxic effects of trehalose. The significance of the presence of trehalase in higher plants, where its substrate has not been found, may lie in the survival value afforded to plants by the conservation of a trehalase gene. We may thus explain the hitherto unknown (9) role of trehalase in higher plants.

The present findings may have implications in understanding the significance of the selection of sucrose rather than trehalose as the translocatory sugar in the higher plants. Arnold (3) reasoned that the translocatory sugar in plants should be a protected (nonreducing) derivative of glucose since the cytoplasm of phloem has glucose-metabolizing enzymes. The protected derivatives of glucose which occur predominantly in nature are sucrose and trehalose. Because of its inherent toxicity to higher plants, trehalose would rule itself out as a candidate. This would probably explain the selection of sucrose, which has the desired properties, as the translocatory sugar in plants.

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## LITERATURE CITED

- ALEXANDER AG 1973 Studies on trehalase in *Saccharum* spp. leaf and storage tissues. *Plant Cell Physiol* 14: 157-168
- ARDITTI J, A DUNN 1969 *Experimental Plant Physiology*. Holt, Rinehart and Winston, Inc., New York, p 265
- ARNOLD WN 1968 The selection of sucrose as the translocate of higher plants. *J Theor Biol* 21: 13-20
- ELBEIN AD 1974 The metabolism of  $\alpha,\alpha$ -trehalose. *Adv Carbohydr Chem Biochem* 30: 227-256
- ERNST R, J ARDITTI, PL HEALEY 1971 Carbohydrate physiology of orchid seedlings. II. Hydrolysis and effects of oligosaccharides. *Am J Bot* 58: 827-835
- FLEISCHMACHER OL, MA VATTUONE, FE PRADO, AR SAMPIETRO 1980 Specificity of sugar cane trehalase. *Phytochemistry* 19: 37-41
- FLEMING ID, HF PEGLER 1963 The determination of glucose in the presence of

- maltose and isomaltose by a stable, specific enzyme reagent. *Analyst* 88: 967-968
8. GLASZIOU KT, KR GAYLER 1969 Sugar transport: occurrence of trehalase activity in sugarcane. *Planta* 85: 299-302
  9. GLASZIOU KT, KR GAYLER 1972 Storage of sugars in stalks of sugarcane. *Bot Rev* 38: 471-490
  10. GUSSIN AES 1972 Does trehalose occur in Angiospermae? *Phytochemistry* 11: 1827-1828
  11. GUSSIN AES, JH MCCORMACK 1970 Trehalase and the enzymes of trehalose biosynthesis in *Lilium longiflorum* pollen. *Phytochemistry* 9: 1915-1920
  12. GUSSIN AES, JH MCCORMACK, LY WAUNG, DS GLUCKIN 1969 Trehalase: a new pollen enzyme. *Plant Physiol* 44: 1163-1168
  13. HUGHES R, HE STREET 1974 Galactose as an inhibitor of the expansion of root cells. *Ann Bot* 38: 555-564
  14. KANDLER O 1967 Biosynthesis of poly- and oligo-saccharides during photosynthesis in green plants. In A San Pietro, FA Greer, TJ Army, eds, *Harvesting the Sun*. Academic Press, New York, p 131
  15. KEEN NT, PH WILLIAMS 1969 Translocation of sugars into infected cabbage tissues during club root development. *Plant Physiol* 44: 748-754
  16. LABAT-ROBERT J, FCH BAUMANN, E BAR-GUILLOUX, D ROBIC 1978 Comparative specificities of trehalases from various species. *Comp Biochem Physiol* 61B: 111-114
  17. LOOMIS WD, J BATTAILE 1966 Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry* 5: 423-438
  18. MAHESHWARI R, C SHAILINI, K VELUTHAMBI, S MAHADEVAN 1980 Interaction of gibberellic acid and indole-3-acetic acid in the growth of excised *Cuscuta* shoot tips *in vitro*. *Plant Physiol* 65: 186-192
  19. MARZULLO G, WF DANFORTH 1969 Ethanol-soluble intermediates and products of acetate metabolism by *Euglena gracilis* var. *bacillaris*. *J Gen Microbiol* 55: 257-266
  20. MATHES MC, M MORSELLI, JW MARVIN 1973 Use of various carbon sources by isolated maple callus cultures. *Plant Cell Physiol* 14: 797-801
  21. MURASHIGE T, F SKOOG 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473-497
  22. NICKELL LG, A MARETZKI 1970 The utilization of sugars and starch as carbon sources by sugarcane cell-suspension cultures. *Plant Cell Physiol* 11: 183-185
  23. OESCH F, H MEIER 1967 Trehalose in the cambial sap of *Fagus sylvatica* L. *Phytochemistry* 6: 1147-1148
  24. POTTY VH 1969 Determination of proteins in the presence of phenols and pectins. *Anal Biochem* 29: 535-539
  25. PRASAD ARS, R MAHESHWARI 1978 Purification and properties of trehalase from the thermophilic fungus *Humicola lanuginosa*. *Biochim Biophys Acta* 525: 162-170
  26. ROBERTS RM, KC TOVEY 1969 Trehalase activity in *Selaginella martensii*. *Arch Biochem Biophys* 133: 408-412
  27. SMITH SE 1973 Asymbiotic germination of orchid seeds on carbohydrates of fungal origin. *New Phytol* 72: 497-499
  28. SOMOGYI M 1952 Notes on sugar determination. *J Biol Chem* 195: 19-23
  29. TREVELYAN WE, DP PROCTER, JS HARRISON 1950 Detection of sugars on paper chromatograms. *Nature (Lond)* 166: 444-445
  30. VERMA DC, DK DOUGALL 1977 Influence of carbohydrates on quantitative aspects of growth and embryo formation in wild carrot suspension cultures. *Plant Physiol* 59: 81-85
  31. YEMM EW, AJ WILLIS 1954 The estimation of carbohydrates in plant extracts by anthrone. *Biochem J* 57: 508-514